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PARTHENOGENESIS IN *CARCINONEMERTES* SPP. (NEMERTEA: HOPLONEMERTEA)

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Abstract

When juveniles of *Carcinonemertes* spp. are removed from male crabs and raised in the laboratory, a 50:50 sex ratio is obtained. Isolated females produce egg strings typical of *Carcinonemertes,* except that the embryos are haploid, with about 13 chromosomes per cell. Larvae develop at least to hatching, and some do hatch. Nearly all of the 146 females so raised produced egg strings. Larvae of all females raised in isolation were haploid while larvae of females put with males were diploid. Females from both U. S. west coast species, *Carcinonemertes epialti* and *C. errans,* and from three hosts, *Cancer magister, C. antennarius,* and *Hemigrapsus oregonensis,* produced these haploid larvae by parthenogenesis.

Introduction

Nemerteans in the genus *Carcinonemertes* live on crabs. Two species of *Carcinonemertes* have been recorded from the U. S. west coast: members of *C. errans* occur on *Cancer magister,* the commercially important dungeness crab (Wickham, 1978), and it is thought that members of *C. epialti* inhabit the other U. S. west coast crabs that harbor worms (Wickham and Kuris, 1985). Most crab species along this coast harbor worms, but the number of species of *Carcinonemertes* that actually occur along this coast is still unknown.

Carcinonemertes juveniles occur on both male and female crabs (Kuris, 1978; Roe, 1979; Wickham, 1980). Male crabs apparently harbor only juvenile worms, since resident worms move from male to female crabs during crab mating (Kuris, 1978; Wickham *et al.,* 1984). Juveniles apparently do not feed, but are maintained by absorbing dissolved organic materials, at least part of which are leaked through the arthrodial membranes of their host's skeleton (Crowe *et al.,* 1982). After a female crab ovulates, her resident worms migrate to the egg clutch (Humes, 1942; Kuris, 1978; Roe, 1979; Wickham, 1980). During the crab's brooding season the worms feed on the developing crab larvae, grow, and reproduce. Each female worm makes several egg strings (Humes, 1942; Wickham, 1979, 1980; Roe, 1984).

Worms can reach enormous numbers on individual hosts, and because they feed on developing zoeae they have been implicated in the decline of the dungeness crab fishery along the U. S. West Coast (Wickham, 1979). Since their feeding biology apparently affects host populations more than other aspects of their lives, most recent studies have focused on these worms as "egg predators" (Roe, 1984; Wickham, 1979; Wickham and Kuris, 1985).

Members of *Carcinonemertes* spp. are definitely predators during their trophic phase on brooding crabs, but most of their life history characteristics are parasitic.

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Parasites have evolved several adaptations to increase reproductive output, including asexual reproduction, increased egg production, hermaphroditism, and parthenogenesis. The present study reports the first evidence for parthenogenesis in *Carcinonemertes.*

Materials and Methods

Worms were raised in isolation (experimental worms) or in groups (controls) in 5-cm diameter plastic disposable petri dishes, either in the constant temperature chamber at California State University, Stanislaus, or on running seawater tables at the Bodega Marine Laboratory. Temperature was maintained close to normal seawater temperature, *i.e.,* 12–14°C. Since it is not known how many species comprise the *Carcinonemertes epialti* group, worms were separated by host. To raise worms, juveniles were removed from male crabs. One juvenile worm was placed with a clump of crab eggs in a petri dish. Similar containers held several worms together as controls. Eggs of the crab *Hemigrapsus oregonensis* were used as food throughout these experiments because brooding females of *H. oregonensis* are collected easily throughout the year. It was previously determined that all worms tested will feed on eggs of *H. oregonensis* (Roe, 1984). Containers were checked every 2–4 days as worms grew. Crab eggs and seawater were changed on a haphazard basis, whenever the eggs or water became fouled or a worm needed more food.

When female worms began laying egg strings in the culture dishes, the egg strings were observed using compound or stereomicroscopes to see if eggs were dividing. In addition, some egg strings of both experimental and control worms were stained with aceto-orcein so that chromosomes in the eggs could be counted. To stain with aceto-orcein, an egg string was removed from the culture dish and put into another 5-cm petri dish containing 3 parts absolute ethanol:1 part glacial acetic acid for at least one hour. The egg string was then placed on a microscope slide with a drop of aceto-orcein (2% aceto-orcein dissolved in 75% acetic acid). A cover slip was quickly placed over the egg string and pressure was applied to compress the eggs and to make some eggs break out of the egg case. These temporary slides were ringed with fingernail polish. The next day the coverslip was removed and a drop of CMCP-9AF (Masters Chemical Co., Inc.), mounting media was placed on the egg string and the cover slip was replaced, to make a permanent slide. All methods to make permanent slides without waiting at least 24 hours resulted in poor chromosome staining.

Stained egg strings were observed under oil at 1000× with a compound microscope and chromosomes were counted.

Results

Juvenile worms placed with crab eggs for food grew to maturity in about 3 weeks (males) to 30 days (females) under the laboratory conditions of these experiments. As seen in Table I, the individuals that grew obtained approximately a 50:50 sex ratio. Females developed slower than males and are about twice the size of males at maturity. These problems can easily account for the slightly fewer females than males in two of the three experimental populations. There is no reason to think that one sex predominated among those worms which, for one reason or another, never grew.

In addition, there was approximately a 50:50 sex ratio (47 females, 43 males) of *C. epialti* on *Hemigrapsus oregonensis* when all worms were counted from 7 ovigerous crabs collected from nature.

Females of both *Carcinonemertes errans* and *C. epialti* produce egg strings even

TABLE I

Number of worms of Carcinonemertes *spp. raised for observations of parthenogenesis*

Species		Number raised			
Worm	Host	Total	Females	Males	Other*
Carcinonemertes errans	*Cancer magister*	320	117	137	66
Carcinonemertes epialti	*Hemigrapsus oregonensis*	75	17	21	37
Carcinonemertes epialti	*Cancer antennarius*	40	12	12	16
		435	146	170	119

* No development, died, etc.

when they are isolated from all other members of their species. Females started making egg strings about 30 days after experiments began. Both experimental and control females laid several egg strings, usually with one to one-and-one-half day intervals between each string. Eggs are 65–75 μm in diameter. An average of 152 ($\pm$108 S.D.) eggs occurred in 14 strings randomly chosen from 71 strings of experimental *C. errans*.

Most egg strings were laid before the eggs had completed meiosis; one and then two polar bodies could be seen during the first one to one-and-one-half hours after an egg string was produced. When egg strings were stained with aceto-orcein during polar body formation, 12 or 13 chromosomes usually could be counted, both in the polar body and remaining in the cell, in both experimental and control worms of both species (Table II, Fig. 1a). From these data it was determined that the haploid number of chromosomes for both *C. errans* and *C. epialti* is probably 13. Polar bodies can be seen through the 2-cell stage in developing worm embryos. However, by this time the chromosomes have started to fragment, clump together, etc.; this can account for cells with both very low and high numbers seen in Table II. In addition, chromosomes often seem to lie on top of one another, and it is sometimes difficult to separate them; this could account for most of the low numbers in both Tables II and III.

Mitotic chromosomes were easiest to count when cells in the egg strings were dividing to the 2-cell stage. After the 4-cell stage, cells became small enough that it was usually difficult to count chromosomes. Early mitotic divisions in embryos of controls of both *C. errans* and *C. epialti* showed the diploid number of 24–26 chromosomes (Table III, Fig. 1b). However, embryos from experimental (isolated) females of both *C. errans* and *C. epialti* showed the haploid number of chromosomes even during mitosis to 2, 4, and later cell stages (Table III, Fig. 1c). A total of 434 cells in approximately 103 egg strings from 35 different experimental females of both species showed that virtually all embryos produced by experimental females were haploid (Table III).

Eggs in egg strings of experimental females developed, at least to the hatching point. When larvae of *Carcinonemertes* hatch they are multicellular and are fully ciliated. Larvae from experimental females appear to be normal except that they seem to have a harder time actually hatching from the egg case than do larvae produced from fertilized eggs. Some larvae from isolated females did hatch, and these larvae lived several days post-hatching, similar to larvae that develop from fertilized eggs of control females.

Although the production of 1N embryos by isolated females of *Carcinonemertes*

TABLE II

Number of cells counted from egg strings of Carcinonemertes *spp. showing the number of meiotic chromosomes listed*

Worm category	Number of chromosomes per cell						
	<10	10	11	12	13	14	15
Experimental[1]							
Carcinonemertes errans (20 egg strings from 13 females)	5	10	26	41	28	11	
Carcinonemertes epialti from *Hemigrapsus oregonensis* (14 egg strings from 9 females)	1	4	7	19	20	1	
Control[2]							
Carcinonemertes errans (6 egg strings)	1	5	8	8	7	2	
Carcinonemertes epialti (9 egg strings)	4	5	8	11	13	2	2

[1] Experimental worms are those raised in isolation.
[2] Control worms are those raised in groups.

spp. has only been observed in laboratory conditions, it does not appear to be a laboratory artifact. Egg strings were produced by 126 of the 146 experimental females. The 20 females that did not produce eggs included worms that died or were removed from observation before they started reproducing, and a few individuals that did not

TABLE III

Number of cells counted from egg strings of Carcinonemertes *spp. showing the number of mitotic chromosomes listed*[1]

Worm group	Number of chromosomes per cell														
	9	10	11	12	13	14	15–19	20	21	22	23	24	25	26	27
Experimental[2]															
Carcinonemertes errans (71 slides from 24 females)	9	30	68	92	101	3					1?	2 + 1?			
Carcinonemertes epialti from *Hemigrapsus oregonensis* (32 slides from 11 females)	2	5	21	43	50	6									
Control[3]															
Carcinonemertes errans (18 slides)							10	8	5	11	10	7	4	4	1
Carcinonemertes epialti (22 slides)			1	2	3		22	7	2	12	6	6	4	2	

[1] Chromosomes of 5 cells/egg string usually counted; 1 egg string per slide in most cases; ½ total number of chromosomes/cell recorded for anaphase and early telophase.
[2] Experimental worms are those raised in isolation.
[3] Control worms are those raised in groups.

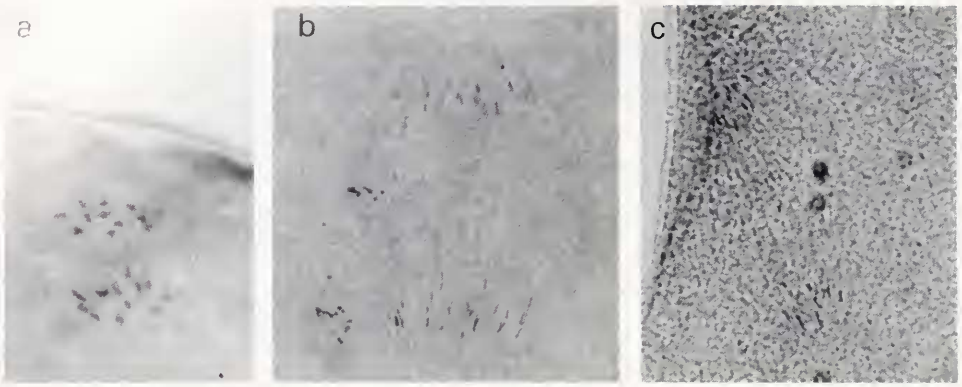

FIGURE 1. a. Polar body formation in egg of control *Carcinonemertes epialti.* Chromosomes nearest edge of the cell are those of the polar body. Some cells showed 12, other cells showed 13 chromosomes during meiotic divisions. Magnification: 1500×. b. Chromosomes in mitotic anaphase during division from one to two cell stage in embryo of control *Carcinonemertes errans.* The small dark spots to the left of the chromosomes are remains of chromosomes in the two polar bodies. Magnification: 1000×. c. Chromosomes in mitotic anaphase during division from two to four cell stage in haploid embryo of experimental *Carcinonemertes errans.* Chromosome number is one-half that of Figure 1 b. Magnification: 1000×. Controls are worms raised in groups. Experimental worms are worms raised in isolation.

begin to feed until many days after the experiments started and therefore were very slow to develop. Some of these females never developed to the point of laying eggs. In addition, of the 440 egg strings of experimental animals observed either with a compound or stereomicroscope to determine if the eggs were dividing, at least 430 strings had dividing, developing eggs. Finally, after two experimental females had made egg strings with haploid embryos, males were added to their culture dishes. Of the eight egg strings checked for chromosome numbers that were made after males were introduced, two egg strings had haploid embryos and six had diploid embryos.

DISCUSSION

These studies show that females of *Carcinonemertes* can produce offspring by parthenogenesis when necessary. That larvae produced by isolated females are indeed produced by parthenogenesis is supported by several lines of evidence. The primary evidence is that these larvae are haploid. In addition, there is no question that these females are indeed females and not self-fertilizing hermaphrodites. Both in cultures and in nature one sees a 50:50 sex ratio. Males and females are easily distinguished (Roe, 1984). Animals were isolated as juveniles, before any gonadal development had occurred. In addition, only juveniles from male crabs were used; all evidence indicates that only juvenile worms inhabit male crabs (Wickham *et al.,* 1984). All lines of evidence indicate that these experimental females produced unfertilized eggs capable of development.

Although the phenomenon of parthenogenetic egg production by *Carcinonemertes* has only been observed in the laboratory, it is thought that parthenogenesis might be a normal part of the life history of these worms. Nearly all 146 females raised for these experiments did produce eggs, and eggs in virtually all 440 strings observed were seen to be dividing and developing. In addition, *Carcinonemertes* often occurs in very low numbers on many crab species (Wickham, in press), and in such

circumstances a female worm would be unlikely to find a mate on her particular host. Parthenogenesis could be a decided adaptive advantage in such situations of low numbers and little mobility.

It remains to be seen whether parthenogenesis in *Carcinonemertes* has ecological significance. A small percentage of parthenogenetically produced larvae do hatch, and appear to behave normally. However, no one has been successful to date in getting any larvae of *Carcinonemertes* spp., normal or parthenogenetic, to settle or grow into juveniles in laboratory conditions. So it is not yet known if parthenogenetic larvae can grow to maturity or if they would be able to reproduce.

A total of 5 apparently diploid larvae were seen in approximately 15,000 cells (430 egg strings × average 152 cells per string) (Table III plus one observation seen before chromosomes were systematically counted from all egg strings). In some of these five cases, the cells appeared in thicker areas on slides, and it could not be determined for certain that cells were not lying on top of one another and chromosomes actually belonged to two cells. However, in most cases the cells appeared to be clearly diploid. In these situations the diploid cells were in embryos that were dividing to 4 cell stage or were already at 4 cell stage. It appears that perhaps in a low percentage of parthenogenetic larvae, the diploid condition is achieved. The mechanism by which diploidy is achieved in this particular situation is not known, but in other parthenogenetic systems, in which meiosis has occurred, it has been found that one or the other polar bodies can fuse with a cell nucleus (Whitfield and Evans, 1983) or the two nuclei of the first mitotic division can fuse (Bell, 1982). It is reasonable to assume that any diploid larvae in the egg strings of parthenogenetic *Carcinonemertes* would have at least an equal chance of hatching as haploid larvae and perhaps a better chance. If these larvae then settled onto a crab and matured, they could reproduce in the normal manner for *Carcinonemertes* and the effort of females to reproduce even in isolation would be an advantage to the population. An apparently similar situation is obtained in all-female populations of the rat schistosome, *Schistosoma douthitti* (summarized in Whitfield and Evans, 1983). In these populations, offspring are produced by parthenogenesis, and most (over 95%) are haploid males. A low frequency of 2N males does occur among the parthenogenetically generated embryos, probably from fusion of the haploid nuclei in early stages of developing embryos. The 1N offspring seem to have low fitness and viability, but the parthenogenetically produced 2N males appear to possess a fitness similar to normally produced offspring.

Although it is by no means limited to parasites, parthenogenesis is a common adaptation of parasites to increase their reproductive output (Price, 1980). Such an adaptation, in animals that show several other traits of parasites as well, characterizes the parasitic nature of *Carcinonemertes*. This idea is supported by negative evidence from the free living nemerteans *Cerebratulus* and *Lineus*. *Cerebratulus* eggs were refractive in response to reagents which readily induce artificial parthenogenesis in some animals (Morse, 1912). Most eggs only developed polar bodies in these experiments, although a few treatments resulted in eggs dividing to the 2 or 4 cell stage; nothing induced development past the early morula stage (Morse, 1912). In studies on *C. lacteus*, Freeman (1978) found that eggs do not develop even the first polar body unless they are fertilized. Unfertilized eggs of *Lineus ruber* can start cleavage, but the cleavage is irregular. Mitoses are multipolar and development is abortive (Langlet, 1972, summarized in Bierne, 1983).

Finally, these studies can be a useful tool in determining the actual number of species of *Carcinonemertes* that actually occur along the U. S. west coast. If males and females from different hosts are raised together, and if only 1N offspring are produced, then we can suspect the worms are different species. However, if worms

from different hosts mate and produce 2N offspring, then we should expect that they are the same species or very closely related, and that environmental conditions determine the morphological differences we see in worms from different hosts.

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